Some fundamental aspects of modelling auxin patterning in the context of auxin-ethylene-cytokinin crosstalk

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Abstract

The activities of hormones in the Arabidopsis root depend on cellular context and exhibit either synergistic or antagonistic interactions. Patterning in Arabidopsis root development is coordinated via a localized auxin concentration maximum in the root tip, mediating transcription of key regulatory genes. Auxin concentration and response are each regulated by diverse interacting hormones and gene expression and therefore cannot change independently of those hormones and genes. For example, experimental data accumulated over many years have shown that both ethylene and cytokinin regulate auxin concentration and response. Using the crosstalk of auxin-ethylene-cytokinin as a paradigm, we discuss the links between experimental data, reaction kinetics and spatiotemporal modelling to dissect hormonal crosstalk. In particular, we discuss how kinetic equations for modelling auxin concentration are formulated based on experimental data and also the underlying assumptions for deriving those kinetic equations. Furthermore, we show that, by integrating kinetic equations with spatial root structure, modelling of spatiotemporal hormonal crosstalk is a powerful tool for analysing and predicting the roles of multiple hormone interactions in auxin patterning. Finally, we summarise important considerations in developing a spatiotemporal hormonal crosstalk model for plant root development.

TEXT

Patterning in Arabidopsis root development is coordinated through a localized auxin concentration maximum in the root tip.[1] Auxin concentration is regulated by diverse interacting hormones and their effects on gene expression.[2-5] This interaction means each cannot change independently of the various crosstalk components in space and time. Important questions for understanding hormonal crosstalk in root development include how hormone concentrations and expression of

the associated regulatory and target genes are mutually related; and how patterning of both hormones and gene expression emerges under the action of hormonal crosstalk. Here we discuss how appropriate kinetics for auxin biosynthesis, degradation and transport can be derived following thermodynamic and kinetic principles. Using the crosstalk between auxin-ethylene-cytokinin as an example, we further consider the links between the experimental data, reaction kinetics and implications for spatiotemporal modelling of hormonal crosstalk.

Kinetics of auxin biosynthesis and degradation

In the absence of transport, equation 1a is the simplest equation to describe the change in auxin concentration.

$$\frac{d[auxin]}{dt} = k_{1b}[S_0] - k_{1d}[auxin] \qquad \text{(equation 1a)}$$

Where $[S_0]$ and $[auxin]$ are the concentration of the precursor for synthesizing auxin and auxin concentration, respectively. k_{1b} and k_{1d} are the biosynthesis and degradation rate constants, respectively, which are related to auxin biosynthesis and degradation gene activity.

At a steady state,

$$[auxin] = \frac{k_{1b}[S_0]}{k_{1d}} \qquad \text{(equation 1b).}$$

Although equation 1b is very simple, it shows that, for a fixed $[S_0]$, steady-state auxin concentration can be changed by altering k_{1b}, k_{1d} or both. This implies that steady-state auxin concentration can be manipulated by modifying auxin biosynthesis, degradation or both. Moreover, increasing k_{1b} or decreasing k_{1d} may lead to the same steady-state auxin concentration.

In Arabidopsis root development, auxin concentration is regulated in part by other hormones influencing its steady state levels.[3,6-9] For example, experimental data show that exogenous application of cytokinin may reduce the endogenous auxin concentration.[6] It can therefore be considered that cytokinin has roles in the negative regulation of auxin biosynthesis, or positive regulation of auxin degradation or both. Moreover, experimental data also show that genes involved in auxin metabolism are differentially expressed in response to altered cytokinin levels and/or responsiveness in Arabidopsis.[10] Thus, we may consider that regulation of auxin concentration by cytokinin is at the transcriptional level. To account for negative regulation of auxin biosynthesis by cytokinin, equation 1a should be modified and becomes equation 2a:

$$\frac{d[auxin]}{dt} = \frac{k_{2b}}{1+\frac{[ck]}{k_{2b1}}}[S_0] - k_{2d}[auxin] \qquad \text{(equation 2a)}$$

where $[ck]$ is the cytokinin concentration and k_{2d} is the auxin degradation rate constant. k_{2b} and k_{2b1} kinetically describe the maximum auxin biosynthesis rate and the degree to which cytokinin negatively regulates auxin biosynthesis. The detailed biological significances of k_{2b} and k_{2b1} depend on the specific mechanism, as discussed below.

Following thermodynamic and kinetic principles,[11-13] equation 2a can be derived by assuming the following mechanism: a) cytokinin negatively regulates the expression of a transcriptional factor; b) the transcriptional factor positively regulates expression of the genes involved in auxin metabolism; c) the increased expression of the genes involved in auxin metabolism increases the enzyme activities for auxin biosynthesis. The term 'transcriptional factor' is used here to represent what may be multiple regulatory proteins working together. Due to these multi-level relationships between cytokinin concentration and its regulation of auxin biosynthesis, k_{2b} and k_{2b1} have

combined information at the transcriptional, translational and metabolic levels. For example, the term $\frac{k_{2b}}{1+\frac{[ck]}{k_{2b1}}}$ in equation 2a as a whole can be considered as the enzyme activity promoting auxin biosynthesis. Thus, for a single enzyme, k_{2b} stands for the maximum enzyme activity, and k_{2b1} describes the binding affinity of cytokinin or its downstream response to a transcriptional factor for down-regulating gene expression of this enzyme. However, since auxin biosynthesis itself comprises multiple enzymes and multiple pathways,[14,15] the enzyme activity for auxin biosynthesis in the format of $\frac{k_{2b}}{1+\frac{[ck]}{k_{2b1}}}$ has already combined the functions of multiple enzymes and multiple pathways. Thus, k_{2b} stands for the apparent maximum activity of multiple enzymes, and k_{2b1} describes the apparent binding affinity at transcriptional level.

After incorporating the negative regulation of auxin biosynthesis by cytokinin, the steady-state auxin concentration is described by equation 2b.

$$[auxin] = \frac{k_{2b}}{1+\frac{[ck]}{k_{2b1}}}\frac{[S_0]}{k_{2d}} \qquad \text{(equation 2b)}$$

Equation 2b describes a negative regulation of auxin concentration by cytokinin, since an increase in cytokinin concentration decreases the steady-state auxin concentration. This relationship is due to a negative transcriptional regulation of the rate of auxin biosynthesis by cytokinin.

An alternative way to realise a negative regulation of auxin concentration by cytokinin is to assume that auxin degradation rate is positively regulated by cytokinin at transcriptional level. For this case, equation 1a becomes equation 3a.

$$\frac{d[auxin]}{dt} = k_{3b}[S_0] - k_{3d}\frac{[ck]}{1+\frac{[ck]}{k_{3d1}}}[auxin] \qquad \text{(equation 3a).}$$

At a steady state,

$$[auxin] = \frac{k_{3b}[S_0]}{k_{3d}}\frac{1+\frac{[ck]}{k_{3d1}}}{[ck]} \qquad \text{(equation 3b).}$$

Equation 3b also describes a negative regulation of auxin concentration by cytokinin, since an increase in cytokinin concentration decreases the steady-state auxin concentration. This relationship is due to a positive regulation of the auxin degradation rate by cytokinin.

Equations 2a and 3a can be combined if a negative regulation of auxin biosynthesis rate by cytokinin and a positive regulation of auxin degradation rate by cytokinin occur simultaneously. This case also results in an overall negative regulation of auxin concentration by cytokinin.
In addition, several hormones in the Arabidopsis root may regulate auxin concentration synergistically or antagonistically.[3,9] For example, auxin biosynthesis can be stimulated by ethylene and inhibited by cytokinins.[6-8,16] Based on the principle discussed above, a positive regulation of auxin concentration by ethylene can also be derived. Moreover, previous research also deduces that the *POLARIS* (*PLS*) gene [17,18] is also required for the correct regulation of auxin concentration.[19] Thus, in the context of auxin-ethylene-cytokinin crosstalk, cytokinin,

ethylene and *PLS* all regulate auxin concentration. By assuming that the regulation of auxin concentration by cytokinin, ethylene and *PLS* is due to the change in the auxin biosynthetic rate, the change in auxin concentration can be described by equation 4a.[20]

$$\frac{d[auxin]}{dt} = \frac{k_{4b1}[ET]}{(k_{4b3} + k_{4b4}[ET])(1 + [CK]/k_{4b2})} \frac{[PLSp]}{(k_{4b5} + [PLSp])} - k_{4d}[auxin] \quad \text{(equation 4a)}$$

Where $[ET]$ and $[PLSp]$ are the ethylene and PLS protein concentration, respectively.

At a steady state,

$$[auxin] = \frac{k_{4b1}[ET]}{k_{4d}(k_{4b3} + k_{4b4}[ET])(1 + [CK]/k_{4b2})} \frac{[PLSp]}{(k_{4b5} + [PLSp])} \quad \text{(equation 4b).}$$

Following our discussion above, all parameters in equations 4a and 4b have biological significance since equation 4a can be derived based on hypothesised regulatory mechanism(s) at a transcriptional level.

For auxin concentration regulation, another level of complexity is that other hormones such as cytokinin and ethylene may also indirectly regulate auxin concentration at the level of metabolic conversion, in addition to their regulation at the level of gene expression. For example, cytokinin-related transcriptional regulation may change the concentration of some metabolites that can be the effectors of auxin metabolism. For this case, kinetic equations can also be derived following thermodynamic and kinetic principles.[11-13]

In summary, auxin biosynthesis and degradation can be regulated by ethylene and cytokinin at both transcriptional and metabolic levels. The formulation of appropriate kinetic equations needs to follow thermodynamic and kinetic principles.[11-13]

Kinetics of auxin transport

In the cytosol, auxin is assumed to be transported by diffusion. However, auxin transport between the cytosol and the cell wall is facilitated by AUX1/LAX influx carriers,[21] and PIN and ABCB auxin efflux carriers located at the plasma membrane.[22,23] Whilst the kinetics of auxin diffusion transport is well established, the kinetics for PIN- or AUX1- facilitated auxin transport can also be derived, as shown previously.[20]

From kinetics to auxin patterning in the context of auxin-ethylene-cytokinin crosstalk

Auxin concentration in the Arabidopsis root is regulated by a hormonal crosstalk network,[19,20,24] consisting of interacting gene expression, signal transduction and metabolic conversions. Since the kinetics of each process in the hormonal crosstalk network and the kinetics of auxin transport can be derived, auxin concentrations in the Arabidopsis root can be calculated. Figures 1 and 2 summarise some key results for a computed auxin concentration using our spatiotemporal hormonal crosstalk model.[20]

---Figures 1 and 2 here---

Figure 1 shows that the modelled auxin concentration can be used to analyse the spatial distribution of auxin at any level of spatial detail, and Figure 2 shows that it can also be used to analyse trends in auxin concentration in different cell types or tissues. Since auxin concentration can be computed spatiotemporally in the Arabidopsis root, modelling of spatiotemporal

hormonal crosstalk is a powerful tool for explaining or interrogating auxin experimental data [19,20,24] and for analysing and predicting the functions of multiple hormone interactions important for auxin patterning.[20]

In general, the appropriate development of a spatiotemporal hormonal crosstalk model requires careful consideration of the following questions: What regulatory relationships among hormones and the associated genes can be derived from experimental data? What kinetic equations can be formulated following thermodynamic and kinetic principles? What level of detail in the spatial root structure is required to be included in the model? What transport kinetics for all hormonal crosstalk components should be formulated? What are the roles of growth in hormonal crosstalk? [25] How can we parameterise a hormonal crosstalk model? [26, 27] The modelling approach with careful consideration of the above questions is likely to prove powerful for dissecting complex regulatory pathways in a range of cell signalling problems.[20,28,29]

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Figure Legends

Figure 1

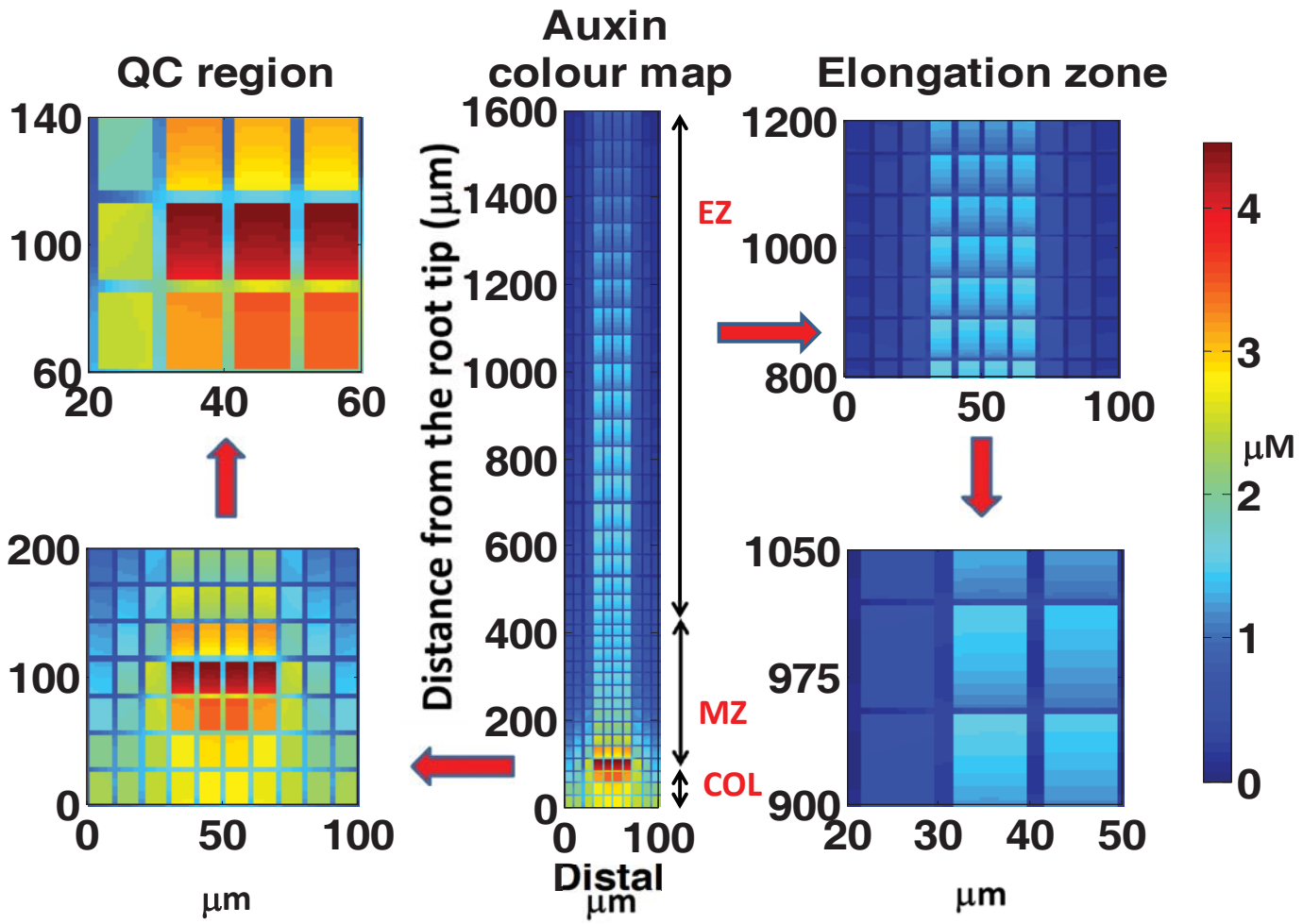
The modelled spatial distribution of auxin in a simplified root map,[20] showing that the auxin maximum is established in the region of the Quiescent Centre (QC). Expanded views in the QC region and the elongation zone show that auxin spatial distribution can be examined at any level

of spatial detail using the spatiotemporal hormonal crosstalk model described recently.[20] EZ, elongation zone. MZ, meristematic zone. COL, columella. Colour bar indicates concentration.

Figure 2

The modelled auxin concentration at different regions or cell types in Arabidopsis root.

A. Auxin profile showing the average auxin concentration along the longitudinal axis of the root with a maximum in the QC region. B. Individual auxin profiles for the epidermal, pericycle and vascular cell files indicate that the auxin maximum is established mainly in the vascular cell files. C. The average auxin concentration in the elongation, meristematic and columella zones, showing that concentrations increase towards the root tip. D. Average auxin concentrations relative to the QC in different regions of the root, showing that the auxin maximum occurs in the QC region and the model can predict the relationship of relative auxin concentration in different regions of Arabidopsis root. QC, quiescent centre; COL, columella; STE, stele; END, endodermis; CO, cortex; EP, epidermis; MZ, meristematic zone; EZ, elongation zone. Figure 2 demonstrates that the spatiotemporal hormonal crosstalk model [20] can compute a variety of auxin concentration data for scrutinising auxin concentrations at different regions or cell types in Arabidopsis root.


QC region
Auxin
colour map
Elongation zone
Distance from the root tip (μm)
EZ
MZ
COL
Distal
μm
μm
μm
μM
140
100
60
20
40
60
200
100
0
0
50
100
1600
1400
1200
1000
800
600
400
200
0
0
100
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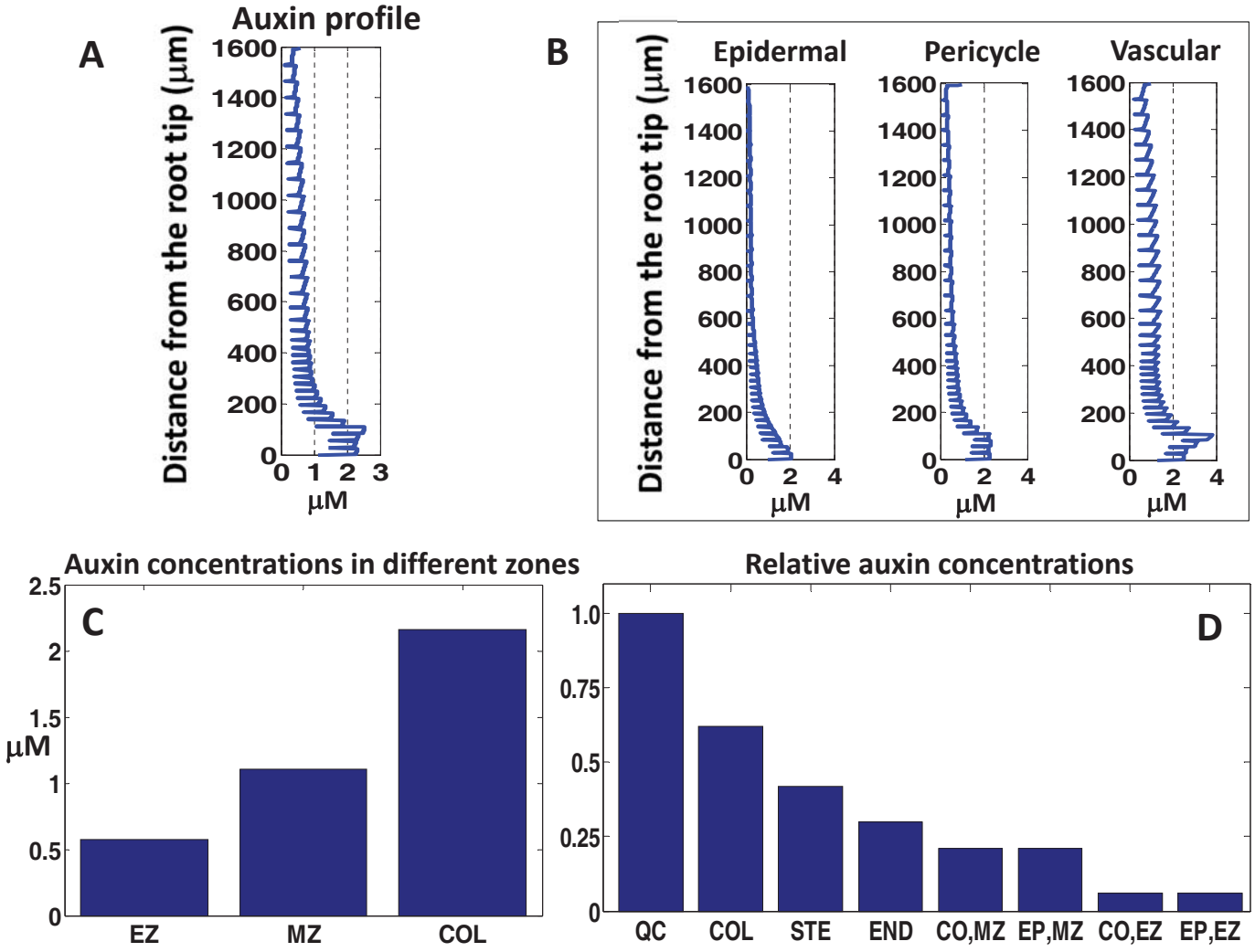
975
900
20
30
40
50
4
3
2
1
0


A
Auxin profile
Distance from the root tip (μm)
1600
1400
1200
1000
800
600
400
200
0
0 1 2 3
μM
B
Epidermal
Pericycle
Vascular
Distance from the root tip (μm)
0 2 4
μM
C
Auxin concentrations in different zones
μM
2.5
2
1.5
1
0.5
0
EZ
MZ
COL
D
Relative auxin concentrations
1.0
0.75
0.5
0.25
0
QC
COL
STE
END
CO,MZ
EP,MZ
CO,EZ
EP,EZ